CLAIMS

1. A photoreactive azido-ruthenium (AzRu) compound which binds to a Ca^{2+}-binding protein.

2. The compound of claim 1, wherein the molar ratio between ruthenium and azide in said compound is 2:1.

3. The compound of claim 1 or claim 2, comprising in its molecule ruthenium, azido group, and chlorine.

4. The compound of claim 3, wherein the molar ratio $Ru:N_3:Cl$ is 2:1:5.

5. The compound of any one of claims 2 to 4, further comprising bound water molecules.

6. The compound of any one of claims 1 to 5, in which one of the atoms is a radioactive isotope.

7. The compound of claim 6, wherein the isotope is ^{103}Ru.

8. The compound of any one of claims 1 to 7, which compound covalently binds to said Ca^{2+}-binding protein following photo-activation by UV irradiation.

9. The compound of claim 8, which binds to the Ca^{2+}-binding site of said Ca^{2+}-binding protein.

10. The compound of any one of claims 1 to 9, which specifically binds to said Ca^{2+}-binding protein, thereby inhibiting its Ca^{2+}-dependent activity.

11. The compound of any one of claims 1 to 10, wherein said Ca^{2+}-binding protein is selected from the group consisting of proteins involved in signal transduction, muscle contraction, neurotransmitter release, hormone secretion, cell motility, apoptosis, fertilization, cell proliferation, cell mitosis and in gene expression; proteins associated with Ca^{2+}-transport, Ca^{2+}-pumps, and with the mitochondrial uniporter; channel protein VDAC; Ca^{2+}-release channel/ryanodine receptor; IP_3 receptor, proteins involved in Ca^{2+}-efflux in mitochondria; and soluble Ca^{2+} binding proteins regulating various cellular activities.

12. The compound of claim 10 or 11, wherein the inhibition of the Ca^{2+}-dependent activity of said Ca^{2+}-binding protein increases by photo-activation, compared to the inhibition without photo-activation.

13. A method of isolating a Ca^{2+}-binding protein from a source comprising the same, which method comprises the steps of:
    i) providing a source comprising a Ca^{2+}-binding protein;
    ii) reacting said source with an AzRu compound of any one of claims 1 to 12, optionally under photo-activation by UV irradiation, whereby said Ca^{2+}-binding protein is bound to said compound;
    iii) isolating the material bound to said compound obtained in step (ii); and
    iv) releasing the Ca^{2+}-binding protein from the product obtained in step (iii).

14. A method according to claim 13, wherein the Ca^{2+}-binding protein is isolated by affinity chromatography.

15. A method according to claim 14, wherein said AzRu compound is bound to particles of porous polymer that are packed in a column, and wherein any Ca^{2+}-binding protein is retained in said column, while other proteins are eluted.

16. A method according to claim 14 or 15, wherein said retained Ca^{2+}-binding proteins are released from the column by calcium ions.

17. A method according to claim 15 or 16, wherein said particles comprise agarose, cellulose, or dextran.

18. A method for characterizing the structure of a Ca^{2+}-binding protein, wherein said Ca^{2+}-binding protein has been isolated by a method according to any one of claims 13 to 17, further comprising a method selected from the group consisting of electrophoresis, autoradiography, liquid chromatography, MALDI-TOF analysis, LC-MS/MS, protein sequencing, and a sequence homology search.

19. A method for the preparation of an AzRu-comprising bio-sensor chip comprising the steps of:

i) providing an azido-ruthenium compound comprising in its molecule ruthenium, azido group, and chlorine at a molar ratio of 2:1:5;

ii) binding said compound to a polymer such as dextran and coupling it to a suitable support, preferably gold-plated surface to give a chip; and

iii) optionally stabilizing the resulting chip.

20. Use of the chip of claim 19 for the isolation of Ca^{2+}-binding proteins, comprising :

i) exposing said chip to a biological sample comprising a Ca^{2+}-binding protein for a time sufficient for binding of the protein with the support-bound ruthenium compound to occur; and

ii) washing said chip with a buffer comprising either calcium or EGTA.

21. Use of the chip of claim 20, comprising surface plasmon resonance.

22. A method of screening for a calcium-binding substances, preferably Ca^{2+}-binding proteins, comprising the steps of:

i) providing test substances, preferably proteins;

ii) contacting said substances with a ruthenium compound as defined in any one of claims 1 to 12 under conditions which allow binding to occur, preferably under UV irradiation;

iii) isolating from the reaction of (ii) those substances that specifically bind to said ruthenium compound; and

iv) releasing the substances obtained in step (iii) from the ruthenium compound by suitable means.

23. The method of claim 22 further comprising the step of testing the substances obtained in step (iv) for their Ca^{2+}-dependent activity.

24. A method according to any one of claims 13 to 18, wherein said AzRu compound is labeled.

25. A method according to claim 24, wherein said labeling is radioactive labeling.

26. A process for preparing a photoreactive azido-ruthenium compound which binds to a Ca^{2+}-binding protein, wherein the molar ratio between ruthenium and azide in said compound is 2:1, comprising:

i) reacting in the dark sodium azide with ruthenium (III) chloride in the presence of HCl;

ii) applying the reaction mixture of the previous step onto a chromatographic column selected from cation-exchanger or hydrophobic;

iii) collecting the fractions having high absorbance at 290 nm; and optionally

iv) drying the collected fractions, redissolving them, rechromatographing them, and optionally crystallizing said compound from methanol.

27. The process of claim 26, wherein the HCl has the concentration in the range of from 0.5 mol/l to 2 mol/l.

28. The process of claim 26, wherein said sodium azide and ruthenium chloride are reacted at about 100°C for about 2 to 4 hrs.

29. The process of claim 26, wherein the product of AzRu migrates as a single spot with Rf being about 0.9 during TLC on cellulose F plates, using 0.16 M ammonium formate, pH 8.5 and 20% methanol as the developer.

30. The process of claim 26, wherein said product is soluble in water, DMF and DMSO, less soluble in methanol, and insoluble in ethanol, ether, chloroform, ethyl acetate, n-butanol, and isopropyl alcohol.

31. The process of claim 26, wherein said product has an absorbance maximum at about 290 nm.

32. The process of claim 26, wherein said product has absorbance of about 15,000 at 290 nm in a water solution, in the concentration of 1.0 M.

33. Use of an AzRu compound of any one of claims 1 to 12 in diagnosing a disorder associated with a defect in the function of a Ca^{2+}-binding protein in a subject, comprising:

i) providing a sample of said subject and a control sample of a normal subject;

ii) contacting said samples with an azido-ruthenium compound as defined in any one of claims 1 to 12 under conditions suitable for binding to occur, preferably under UV irradiation;

iii) isolating from the mixtures obtained in ii) ruthenium-bound substances; and

iv) comparing the said substances obtained in iii) for said sample with the substances obtained in step iii) for said control sample;

whereby a difference between the substances obtained in said sample and said control sample indicates a possible disorder in Ca^{2+}-binding protein in said patient.

34. Use of an AzRu compound of any one of claims 1 to 12 in the preparation of a medicament for treating a disorder associated with a defect in the function of a Ca^{2+}-binding protein.

35. A pharmaceutical composition containing an AzRu compound, or a solvate thereof, prepared by a process of any one of claims 26 to 32.

36. A pharmaceutical composition according to claim 35, further comprising a carrier, stabilizer, adjuvant, diluent, or excipient.

37. A pharmaceutical composition according to claim 35, for use as a medicament for treating or preventing a disorder associated with a defect in the function of a Ca^{2+}-binding protein.

38. A pharmaceutical composition according to claim 37, for inhibiting the calcium-binding activity of said Ca^{2+}-binding protein.

39. A pharmaceutical composition according to claim 35, further inhibiting apoptotic or necrotic cell death.